Importance of Chloramphenicol Determination

Antibiotic residues in foods pose a serious threat to public health. Human use of Chloramphenicol, a broad-spectrum antibiotic, is found primarily in developing countries due to its low cost. The use of Chloramphenicol in developed nations is generally limited to topical applications for the treatment of eye infections; as chloramphenicol can adversely affect bone marrow, causing aplastic anemia, which is usually fatal. Oral chloramphenicol treatment is therefore only considered appropriate for the treatment of MRSA or other highly antibiotic resistant infections. Chloramphenicol is more frequently used in the veterinary treatment of infections in small mammals and also in amphibians to treat chytridiomycosis, a fungal disease responsible for the loss of one-third of all species of frogs within the past 30 years. The use of Chloramphenicol in food-producing animals is prohibited in many countries including the United States, Canada, the European Union, and Australia due to the high potential risk of severe effects such as aplastic anemia, allergic reactions, and the promotion of antibiotic resistance. The U.S., Canada, and the EU have also imposed bans on all imported foods containing Chloramphenicol residues. The monitoring of water sources and food products, such as meat, milk and honey, for antibiotic residues is necessary to ascertain that these compounds are not misused and do not present a danger to human or animal health.

The Abraxis Chloramphenicol ELISA allows the determination of 40 samples in duplicate determination. Only a few milliliters of sample are required. The test can be performed in less than 2 hours.

Performance Data

Test sensitivity: The limit of detection for Chloramphenicol calculated as Xn +/- 3SD (n=20) or as 90% B/Bound is equal to 0.023 ng/mL. The concentration of residue necessary to cause 50% inhibition (50% B/B) is approximately 0.44 ng/mL. Determinations closer to the middle of the calibration range of the test yield the most accurate results.

Test reproducibility: Coefficients of variation (CVs) for standards: <10%; CVs for samples: <15%.

Selectivity: This ELISA recognizes Chloramphenicol and related compounds with varying degrees:

- Chloramphenicol 100%
- Chloramphenicol glucuronide 25%
- Thiophenicol <1%
- Florphenicol <1%

Samples: To eliminate matrix effects in fish, shrimp, honey, and milk samples, sample clean-up may be required. See Preparation of Samples section.

General Limited Warranty: Abraxis LLC warrants the products manufactured by the Company against defects and workmanship when used in accordance with the applicable instructions for a period not to extend beyond the product’s printed expiration date. Abraxis makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.

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**B. Test Preparation**

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. We recommend using a multi-channel pipette or a stepping pipette for adding the conjugate, antibody, substrate and stop solutions in order to equalize the incubations periods of the solutions on the entire microtiter plate. Please use only the reagents and standards from one package lot in one test, as they have been adjusted in combination.

1. Adjust the microtiter plate and the reagents to room temperature before use.
2. Remove the number of microtiter plate strips required from the foil bag. The remaining strips are stored in the foil bag and zip-locked closed. Store the remaining kit in the refrigerator (4-8°C).
3. The standard solutions, substrate, and stop solutions are ready to use and do not require any further dilutions.
4. The antibody provided is lyophilized (3 vials). Before each assay, calculate the volume of antibody needed (when reconstituted, each vial will provide enough antibody for approximately 65 wells). Reconstitute only the amount necessary for the samples to be analyzed. Once reconstituted, the antibody solution will only remain viable for 1 week (store refrigerated). If additional samples are to be analyzed greater than one week from reconstitution, a new vial of antibody will need to be prepared. To reconstitute, add 3.5 mL of Antibody Diluent to each vial of antibody required and vortex thoroughly.
5. The conjugate provided is lyophilized (3 vials). Before each assay, calculate the volume of conjugate needed (when reconstituted, each vial will provide enough conjugate for approximately 65 wells). Reconstitute only the amount necessary for the samples to be analyzed. Once reconstituted, the conjugate solution will only remain viable for 1 week (store refrigerated). If additional samples are to be analyzed greater than one week from reconstitution, a new vial of conjugate will need to be prepared. To reconstitute, add 3.5 mL of Conjugate Diluent to each vial of conjugate required and vortex thoroughly.
6. Dilute the wash buffer concentrate at a ratio of 1:5. If using the entire bottle (100 mL), add to 400 mL of deionized or distilled water.
7. Dilute the Sample Diluent concentrate (10X) at a ratio of 1:10. If using the entire bottle (25 mL), add to 225 mL of deionized or distilled water.
8. The stop solution should be handled with care as it contains diluted H2SO4.

**C. Assay Procedure**

1. Add 50 µL of the standard solutions and samples or sample extracts into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.
2. Add 50 µL of reconstituted enzyme conjugate solution to the individual wells successively using a multi-channel pipette or a stepping pipette.
3. Add 50 µL of reconstituted antibody solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for about 30 seconds. Be careful not to spill contents.
4. Incubate the strips for 20-30 minutes at room temperature. Protect the strips from direct sunlight.
5. After incubation, remove the covering and vigorously shake the contents of these wells into a sink. Wash the strips three times using the 1X washing buffer solution. Use at least a volume of 250 µL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
6. Add 150 µL of substrate (color) solution to the wells. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for about 30 seconds. Incubate the strips for 20-30 minutes at room temperature. Protect the strips from direct sunlight.
7. Add 100 µL of stop solution to the wells in the same sequence as for the substrate solution.
8. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

**D. Evaluation**

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (4-Parameter preferred) or Logit/Log. For manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the %B/B0 for each standard by dividing the mean absorbance value for each of the standards by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the %B/B0 for each standard on the vertical (y) axis versus the corresponding Chloramphenicol concentration on the horizontal logarithmic (x) axis on graph paper. %B/B0 for samples will then yield levels in ppb of Chloramphenicol by interpolation using the standard curve. Samples showing lower concentrations of Chloramphenicol compared to Standard 1 (0.025 ng/mL) are considered as negative. Samples showing a higher concentration than Standard 7 (2.0 ng/mL) must be diluted further to obtain accurate results.

**E. Additional Materials**

1. Micro-pipettes with disposable plastic tips (10-200 and 200-1000 µL)
2. Multi-channel pipette (10-250 µL) or stepping pipette with plastic tips (10-250 µL)
3. Microtiter plate reader (wave length 450 nm)
4. Timer
5. Tape or Parafilm

**F. Working Scheme**

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

**G. Preparation of Samples**

**Fish/Shrimp Extraction**

1. Weigh 3g of homogenized fish or de-shelled shrimp (should have a paste-like consistency) into a 10mL or 225 mL glass vial with a Teflon-lined cap.
2. Add 3 mL of Ethyl Acetate. Vortex thoroughly. Mix using an overhead tube rotator for 10 minutes.
3. Centrifuge vial for 10 minutes at 3000 g. Pipette 4 mL of the supernatant (top layer) into a clean vial.
4. Evaporate to dryness at 40-60°C under a gentle stream of nitrogen.
5. Add 1 mL of Iso-octane / Trichloromethane (2:3) and vortex thoroughly to re-dissolve.
6. Add 2 mL of Sample Diluent (1X) and vortex thoroughly. Centrifuge vial for 10 minutes at 4000 g.
7. Pipette supernatant (top layer) into a clean vial. This will then be analyzed as sample (Assay Procedure, step 1).

**Honey Sample Extraction**

1. Add 3 g of honey to a clean glass vial with a Teflon-lined cap.
2. Add 3 mL of distilled or deionized water. Vortex.
3. Add 6 mL of Ethyl Acetate. Vortex. Mix using an overhead tube rotator for 10 minutes.
4. Centrifuge vial for 10 minutes at 3000 g. Pipette 4 mL of the supernatant (top layer) into a clean vial.
5. Evaporate to dryness at 40-60°C under a gentle stream of nitrogen.
6. Add 1 mL of Iso-octane / Trichloromethane (2:3) and vortex thoroughly to re-dissolve.
7. Add 1 mL of Sample Diluent (1X) and vortex thoroughly. Centrifuge vial for 10 minutes at 3000 g.
8. Pipette supernatant into a clean vial. This will then be analyzed as sample (Assay Procedure, step 1).

The ELISA result will show the Chloramphenicol concentration contained in the fish/shrimp samples (no correction factor is necessary). Highly contaminated samples (those outside of the calibration range of the assay) must be diluted and re-analyzed.

**Milk**

No sample extraction is necessary for the analysis of milk samples. Proceed to Assay Procedure, step 1. Highly contaminated samples (those outside of the calibration range of the assay) will need to be diluted and re-analyzed.

For additional extraction procedures for various matrices please contact Abraxis LLC.